VITROS Products Chemistry

INSTRUCTIONS FOR USE

VITROS ALKP Slides

ALKP

Alkaline Phosphatase

Intended Use

For in vitro diagnostic use only.

VITROS ALKP Slides quantitatively measure alkaline phosphatase (ALKP) activity in serum and plasma.

Summary and Explanation of the Test

Alkaline phosphatase is present mainly in bone, liver, kidney, intestine, placenta, and lung. Serum alkaline phosphatase may be elevated in increased bone metabolism, for example, in adolescents and during the healing of a fracture; primary and secondary hyperparathyroidism; Paget's disease of bone; carcinoma metastatic to bone; osteogenic sarcoma; and Hodgkin's disease if bones are invaded. Hepatobiliary diseases involving cholestasis, inflammation, or cirrhosis increase alkaline phosphatase activity; alkaline phosphatase activity may be increased in renal infarction and failure and in the complications of pregnancy. Low alkaline phosphatase activity may occasionally be seen in hypothyroidism. [1]

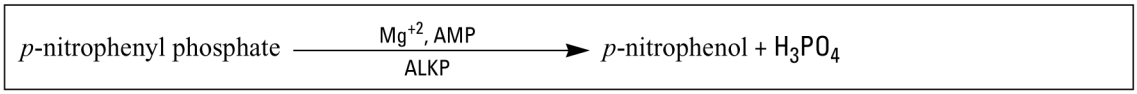
Principles of the Procedure

The VITROS ALKP Slide is a dry, multilayered, analytical element coated on a polyester support.

An 11 μL drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. The spreading layer contains the p-nitrophenyl phosphate substrate and other components needed for the reaction. The ALKP in the sample catalyzes the hydrolysis of the p-nitrophenyl phosphate to p-nitrophenol at alkaline pH. The p-nitrophenol, which absorbs light at wavelengths in the region of 400 ηm, diffuses into the underlying layer, and it is monitored by reflectance spectrophotometry. The rate of change in reflection density is converted to enzyme activity.

Test Type	Wavelength	Assay Time and Temperature
Multiple-point rate	400 ηm	Approximately 5 minutes at 37°C

Reaction Sequence

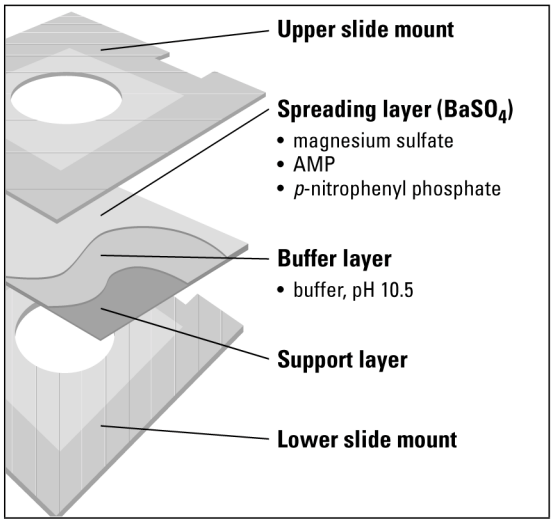


Reagents

Slide Ingredients

Reactive ingredients are p-nitrophenyl phosphate; 2-amino-2-methyl-1-propanol (AMP); and magnesium sulfate. Other ingredients include pigment, binders, buffers, surfactants, cross-linking agent, and stabilizer.

Slide Diagram



Slide Labeling

The cartridge's outer carton is labeled with the test name, slide lot number, expiration date, and required storage temperature.

Slide Cartridge Handling

CAUTION:	**Protect the inner wrapper from damage before opening.**

- Do not drop a case of cartridges.
- Do not cut into the inner wrapper with a sharp instrument when opening the case.

Slide Storage

Unopened slide cartridges:

Store at or below 2°–8°C (36° –46°F).

Cartridges in the system's slide supply:

- Leave in the slide supply for no more than two weeks, then replace with a fresh cartridge.
- Leave in the slide supply when the system is turned off for up to two hours.
- Verify performance with control materials:
  - If the system is turned off for more than two hours
  - After reloading cartridges that have been removed from the slide supply and stored for later use

Slide Stability

VITROS ALKP Slides are stable until the expiration date on the carton when they are stored and handled as specified.

Slide Preparation

- Remove slide cartridges from storage.
- The slide cartridge must reach room temperature, 18°–28°C (64°–82°F), before it is unwrapped and loaded into the slide supply. Allow the cartridge to warm up at least:
  - 60 minutes after removing from the freezer

    or
  - 30 minutes after removing from the refrigerator
- Remove the inner wrapper and immediately load into the slide supply.

NOTE: Load the cartridges within 24 hours after they reach room temperature.

Specimen Collection and Preparation

Patient Preparation

No special patient preparation is necessary.

Recommended Specimen Types

Serum; heparin plasma.

Specimens Not Recommended

EDTA, citrate, and fluoride/oxalate should not be used as anticoagulants.[2]

Specimen Collection and Preparation

- Collect specimens using standard laboratory procedures.[3, 4]
- Refer to the operator's manual section on sample handling for recommended minimum specimen volumes for your system.
- Centrifuge specimens and remove the serum from the clot within 4 hours of collection.[5]

Handling and Storage Conditions

- Handle specimens as biohazardous material.
- Handle specimens in stoppered containers to avoid contamination and evaporation.
- Storage requirements:[6]
  - Store at room temperature or below up to 4 days

Testing Procedure

Materials Required But Not Provided

The following items are required to perform the test for ALKP:

- VITROS Chemistry Calibrator Kit 3
- Quality-control materials, such as VITROS Performance Verifiers
- For dilution, VITROS 7% BSA

Operating Instructions

Refer to the operator's manual for complete instructions on operation of your system.

Sample Dilution

If samples show alkaline phosphatase activities that exceed the system's reportable (dynamic) range, follow this procedure.

1. Dilute sample with VITROS 7% BSA.
2. Reanalyze.
3. Multiply the results by the dilution factor to obtain the original sample's ALKP activity.

Calibration

Required Calibrators

VITROS Chemistry Calibrator Kit 3

Calibrator Preparation, Handling, and Storage

Refer to the calibrator package insert for information about reconstitution and use of the Chemistry Calibrator Kit.

Calibration Procedure

Refer to the calibration section of your operator's manual.

When to Calibrate

- Calibrate when the slide lot number changes.
- Calibrate when critical system parts are replaced due to service or maintenance.
- If quality-control results are consistently outside acceptable limits, calibration might be required. Refer to your operator's manual for more detail.
- Calibrate when government regulations require. In the US, CLIA regulations require calibration or calibration verification at least once every six months.

Reference Method

Calibration is traceable to the alkaline phosphatase reference method proposed by Bretaudiere.[7]

Calibration Model

Multiple-point rate (described in your operator's manual).

Quality Control

Procedure Recommendations

- Handle quality-control materials as biohazardous material.
- Analyze quality-control materials in the same manner as patient samples, before or during patient sample processing.
- Analyze control materials at least once per day to verify system performance.
- Choose control levels that check the clinically relevant range.
- Refer to the quality control section in your operator's manual for additional information on quality-control procedures for VITROS Systems.
- Refer to Internal Quality Control Testing: Principles and Definitions for general quality-control recommendations.[8]

Quality-Control Material Selection

- VITROS Performance Verifiers are specially formulated for use with VITROS Systems.
- Other control materials may show a difference when compared with other alkaline phosphatase methods if they:
  - Depart from a true human serum/plasma matrix
  - Contain high concentrations of preservatives, stabilizers, or other nonphysiological additives
- Enzyme activity might also vary with enzyme source, diluent temperature, and activation time during reconstitution.
- Do not use control materials stabilized with ethylene glycol.

Quality-Control Material Preparation and Storage

Refer to the manufacturer's product literature for preparation, storage, and stability information.

Expected Values and Reporting Results

Reference Interval

Conv./SI Units (U/L)	Alternate Units (μkat/L)
38–126	0.6–2.1

This reference interval is the central 95% of results from an internal study of 273 apparently healthy adults from a working population (154 females and 119 males). Each laboratory should verify the validity of this interval for the population it serves.

Reporting Units and Unit Conversion

Conventional and SI Units	Alternate Units
U/L	μkat/L (U/L x 0.0167)

Limitations of the Procedure

Known Interfering Substances

The VITROS ALKP method was screened for interfering substances. The following substances, when tested at the concentrations indicated, caused the bias shown.

	Conventional and SI Units			Alternate Units		
Interferent*	Interferent Concentration	Analyte Activity (U/L)	Average Bias (U/L)	Interferent Concentration (μmol/L)	Analyte Activity (μkat/L)	Average Bias (μkat/L)
Bilirubin	20 mg/dL	138	+31	342	2.3	+0.52
Methotrexate	200 μg/mL	150	+27	440	2.5	+0.45
Nitrofurantoin	40 μg/mL	150	+27	168	2.5	+0.45

* It is possible that other interfering substances may be encountered. These results are representative; however, your results may differ somewhat due to test-to-test variation. The degree of interference at concentrations other than those listed might not be predictable.

Other Limitations

- Some drugs that have significant light absorbance in the region of 400 ηm can cause a spectral interference.
- Some drugs and patient conditions are known to alter alkaline phosphatase activity in vivo. A compilation of this information is available in the literature. [9, 10]

Performance Characteristics

Reportable Range (Dynamic Range)

Conv./SI Units (U/L)	Alternate Units (μkat/L)
20–1500	0.33–25.05

Refer to Sample Dilution under "Testing Procedure" for out-of-range samples.

Sensitivity

The lower limit of the reportable (dynamic) range is 20 U/L (0.33 μkat/L).

Precision

Precision was evaluated with quality-control materials on VITROS 250, 700, and 950 Chemistry Systems following NCCLS Protocol EP5-T2. [11]

These results are guidelines. Variables such as instrument maintenance, environment, slide handling/storage, control material reconstitution, and sample handling can affect the reproducibility of test results.

ALKP Precision

	Conventional/SI Units (U/L)			Alternate Units (μkat/L)			Within		
SYSTEM	Mean Activity	Within Day SD*	Within Lab SD**	Mean Activity	Within Day SD*	Within Lab SD**	Lab CV%**	No. Observ.	No. Days
VITROS 250	74	0.7	1.1	1.2	0.01	0.02	1.4	79	20
	469	5.2	7.4	7.8	0.09	0.12	1.6	78	20
VITROS 700	78	2.2	3.5	1.3	0.04	0.06	4.5	124	23
	135	3.6	5.7	2.3	0.06	0.10	4.2	88	23
	270	5.0	7.7	4.5	0.08	0.13	2.9	120	23
	1066	21.1	27.8	17.8	0.35	0.46	2.6	86	23
VITROS 950	83	1.4	1.8	1.4	0.02	0.03	2.1	84	23
	491	9.1	11.7	8.2	0.15	0.20	2.4	86	23

* Within Day precision was determined using two runs/day with two to three replications.

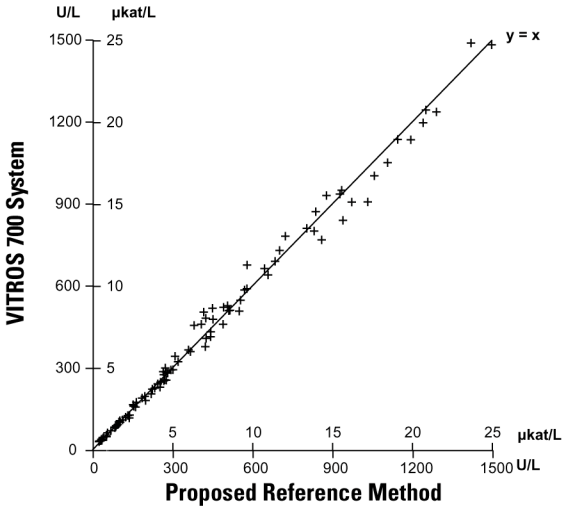
** Within Lab precision was determined using a single lot of slides and calibrating weekly.

Accuracy

The plot and table show the results of a comparison of serum specimens analyzed on the VITROS 700 System with those analyzed using the proposed alkaline phosphatase reference method. Testing followed NCCLS Protocol EP9-A. [12]

The table also shows the results of comparisons of the VITROS 250 and 950 Systems with the VITROS 700 System.

ALKP/Serum



Method Comparison (Serum)

				Conventional/SI Units (U/L)			Alternate Units (µkat/L)		
	n	Slope	Correlation Coefficient	Range of Sample Activity	Intercept	Sy.x	Range of Sample Activity	Intercept	Sy.x
700 System vs. reference method	94	0.98	0.991	26–1477	6.9	34.4	0.4–24.7	0.12	0.58
250 System vs. 700 System	87	0.97	0.999	50–1493	2.6	12.1	0.8–24.9	0.04	0.20
950 System vs. 700 System	117	1.01	0.999	31–1436	0.7	9.4	0.5–24.0	0.01	0.16

Specificity

The following substances were tested with VITROS ALKP Slides and found not to interfere (bias < 13 U/L):

Compound	Concentration
Ascorbic acid	3 mg/dL
Bilirubin	5 mg/dL
Theophylline	20 µg/mL

Compound	Concentration
Total protein	10 g/dL
Urea nitrogen	100 mg/dL

References

1. Tietz NW (ed). Fundamentals of Clinical Chemistry. ed. 3. Philadelphia: WB Saunders; 385–386; 1987.
2. Clinical Laboratory Handbook for Patient Preparation and Specimen Handling. Fascicle VI: Chemistry/Clinical Microscopy. Northfield, IL: College of American Pathologists; 1992.
3. NCCLS. *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture*. NCCLS Document H3-A3. Wayne, PA: NCCLS; 1991.
4. NCCLS. *Procedures for the Collection of Diagnostic Blood Specimens by Skin Puncture*. NCCLS Document H4-A3. Wayne, PA: NCCLS; 1991.
5. Clinical Laboratory Handbook. Fascicle VI. Refer to reference 2 for complete citation.
6. Clinical Laboratory Handbook. Fascicle VI. Refer to reference 2 for complete citation.
7. Bretaudiere J, et al. Criteria for Establishing a Standardized Method for Determining Alkaline Phosphatase in Serum. Clin. Chem. 23:2263; 1977.
8. NCCLS. *Internal Quality Control Testing: Principles and Definitions*. NCCLS Document C24-A. Wayne, PA: NCCLS; 1991.
9. Young DS. Effects of Drugs on Clinical Laboratory Tests. ed. 4. Washington, D.C.: AACC Press; 1995.
10. Friedman RB, Young DS. Effects of Disease on Clinical Laboratory Tests. Washington, D.C.: AACC Press; 1990.
11. NCCLS. *User Evaluation of Precision Performance with Clinical Chemistry Devices*. NCCLS Document EP5-T2. Wayne, PA: NCCLS; 1992.
12. NCCLS. *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline*. NCCLS Document EP9-A. Wayne, PA: NCCLS; 1995.

Revision History

Date of Revision:	Version:	Description:
2002APR19	1.0	New format, technically equivalent to 11/96.

When this Instructions For Use is replaced, sign and date below and retain as specified by local regulations or laboratory policies, as appropriate.

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Signature Obsolete Date

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